

## Impacts of whey protein edible coating on chemical and microbial factors of gutted kilka during frozen storage

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Received: September 2009

Accepted: December 2009

### Abstract

The aim of the present study was to investigate the impact of whey protein coating on quality and shelf life of Kilka fish. Whey protein edible coating was prepared in three different concentrations, including 3, 7, 10 and 13%. Then gutted Kilkas were coated for 1h, packed in polyethylene dishes with cellophane blanket and stored at -18 °C. Total microbial count, total volatile nitrogen (TVN) and moisture evaluation were carried out within 0, 1, 2, 3 and 4 months (sample size: 75 packages, weighted 250 grams each). Results showed that there was no significant difference between total microbial count and total volatile nitrogen among samples ( $p>0.05$ ). Moisture of coated samples with 13% concentration of whey protein had significant difference with other treatments ( $p<0.05$ ) suggesting that whey protein edible coating with 13 % concentrations can enhance quality and increase shelf life of Kilka fish in storage of freezing up to 4 months.

**Keywords:** edible films and coating, whey protein, Kilka fish, Shelf life

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## Introduction

Fish is one of the most important protein sources and other nutrients in many parts of the world and some countries rely heavily on fish export for their income (Mahboob *et al.*, 2009). Clupeids, also known as kilka, including anchovy (*Clupeonella engrauliformis*), common kilka (*C. cultriventris*), and big-eyed kilka (*C. grimmi*), are one of the most abundant fish in the Caspian Sea (Fazli *et al.*, 2007). Freezing is a common preservation method using control or decrease biochemical changes in fish that occur during storage. However, frozen storage does not completely inhibit chemical reactions (e. g., lipid oxidation), that lead to quality deterioration of fish. Preservatives such as phosphates are often applied to seafood products, to improve their shelf life. Antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been commonly used by food industry to improve product quality during storage, resulting in increased product shelf life. To meet consumer demands for safer foods, numerous studies are currently focused on using natural ingredients to enhance food quality and shelf life to avoid the use of synthetic preservative (Sathivel, 2005). Edible films and coatings, as well as biodegradable materials offer alternative packaging systems, which may replace some synthetic packaging materials or reduce the use of synthetic materials by partially replacing those (Regalado *et al.*, 2006).

An edible film is generally defined as a thin layer of edible material formed on a food as a coating or placed on or between food components. This film can be applied by immersion, spraying and or panning (Regalado *et al.*, 2006). Edible films and coatings are intended to help maintain the quality and shelf life of food products by controlling the transfer of moisture, oxygen, carbon dioxide, lipids, aromas, flavors, and food additives (Sothornvit & Krochta, 2005). Polysaccharides or proteins can be used to coat fish fillets to suppress quality changes during frozen storage. Some edible coatings applied on frozen foods act as barriers to control moisture transfer (Sathivel, 2005). Whey is the yellow-green liquid that separates from the curd during manufacture of cheese and casein. Whey protein concentrate (WPC) is produced by an industrial fractionation process involving ultra-filtration and dia-filtration of pasteurized liquid whey. Whey protein has also some functional properties of interest to the food industry, such as solubility, emulsification, foaming, gelation, and viscosity development (Regalado *et al.*, 2006). The formation of edible films and the coating from whey proteins can increase the utilization of whey, improve the nutritional values of foods and prolong shelf life (Ozdemir & Floros, 2008). Research on shelf life extension of fish by edible coating has so far been limited (Piayachomkwan & Penner, 1995, Min *et al.*, 2005, Shah *et al.*, 1999,

Stuchell & Krotcha, 1995, Kilincerker *et al.*, 2009, Sathivel, 2005 and Crapo *et al.*, 1999). In Iran, Mirhashemi Rostami (2000) used coconut oil to coat *Acipenser persicus* fillets. No researches have been published on whey protein edible coating applied to seafood in Iran. The aim of this study was to investigate the effect of whey protein coating on moisture loss, microbial characteristic and enhancing shelf life of coated Kilka during frozen storage.

### Materials and methods

This study was conducted in the National Fish Processing Research Center (Iran, Bandaranzali). WPC (prepared from Kheizaran Food Industries Co.) used to prepare edible coating according to Perez-Gago *et al.* (1999) method.

Coating formulation derived from Different concentration of whey protein solutions including 3%, 7%, 10% and 13% (w/w) were prepared, according to Mor and Sheomaker, (1999), Franssen *et al.* (2004) and Perez-Gago *et al.* (2006), by breaking up 600, 1400, 2000 and 2600 g whey protein concentrate in separate tanks with 20 liter water. The quantity of used gutted Kilka fish for preparation experimental and control treatment, according to 3 Repetitions for each test, has spotted 75 packages and each weighted 250 g.

Fresh fish has provided from quay of Bandar Anzali, in Guilan Province. Fish were carefully gutted and dressed by hand, and then were divided into two groups, including 4kilos of

untreated gutted Kilka fish as blank control, which immediately packed in polyethylene dishes with cellophane blanket and stored at -18°C for 4 month and 15kilos of gutted Kilka fish immersed in whey protein solution with nominative concentration for 1h. Samples in the latter 2group were kept in baskets about 1 min to dissenting surplus of solution from fish surfaces. After packaging all samples in polyethylene dishes with cellophane blanket, they were moved to a -18°C freezer. There were 5 treatments in the present study, including treatment 1 (uncoated Kilka fish), treatment 2 (coated fish with 3% WPC), treatment 3 (coated with 7% WPC), treatment 4 (coated with 10% WPC) and treatment 5 (coated with 13% WPC).

Sampling were carried out in 5 phases, numbered as 0 to 4 one day after storage, followed by 1, 2, 3 and 4 month. For total microbial count, treatments were prepared according to standard no. 356 and total bacteria counting have done according to standard no. 2394-1 (Iranian National Standard 356, 1997; Iranian National Standard 2394-1, 2000). Moisture (Parvaneh, 1998) and TVN has measured by Kjeldahl titration method (Pearson, 1997). Lee method was used to determine amount of peroxide (Pearson, 1997). To statistically analyze data, One-way ANOVA was applied and the mean values were compared using Tukey test at reliability level of 5% and SPSS software, 16.0.

## Results

The initial total microbial count in each pack was 2.31 log CFU/g, followed by an increase as 2.46, 2.87, 3.80 and 5.08 log CFU/g during phases 1, 2, 3 and 4 respectively in uncoated samples ( $p < 0.05$ ). Similarly, coated samples had significant difference in total microbial counts, after 0, 1, 2, 3 and 4 month

freezing storage at  $-18^{\circ}\text{C}$  ( $p < 0.05$ ). There was no significant difference ( $p > 0.05$ ) among total microbial counts in whey protein coated samples (Fig. 1).

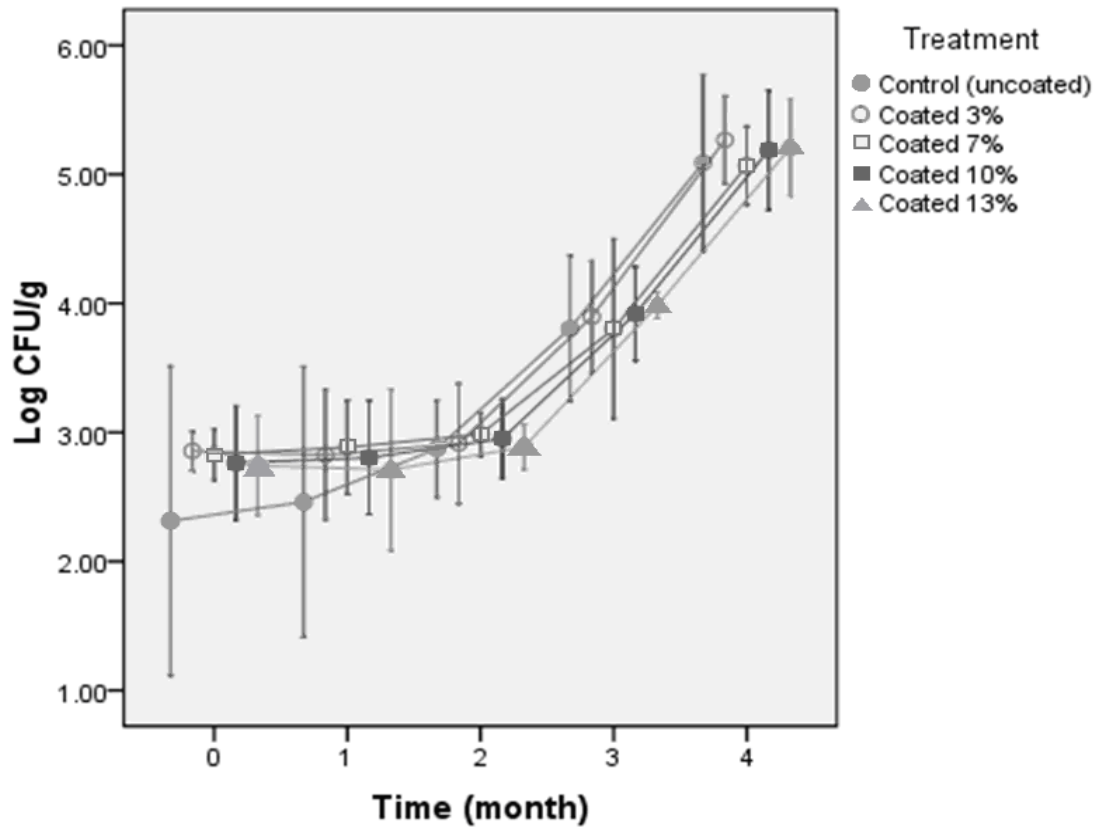


Figure 1: Total microbial counts of coated and uncoated samples with whey protein During a 4-month frozen storage

During storage, the initial total volatile nitrogen was 11.25 mg/100g muscle after 1 day storage, and then 11.73 to 17.72 mg/100g muscle at the end of 4 month storage for uncoated samples ( $p < 0.05$ ). Similarly, coated samples had significant difference

( $p < 0.05$ ) in total volatile nitrogen, after 0, 1, 2, 3 and 4 month freezing storage (Fig. 2). Total volatile nitrogen (TVN) did not show any significant difference in whey protein coated samples ( $p > 0.05$ ).

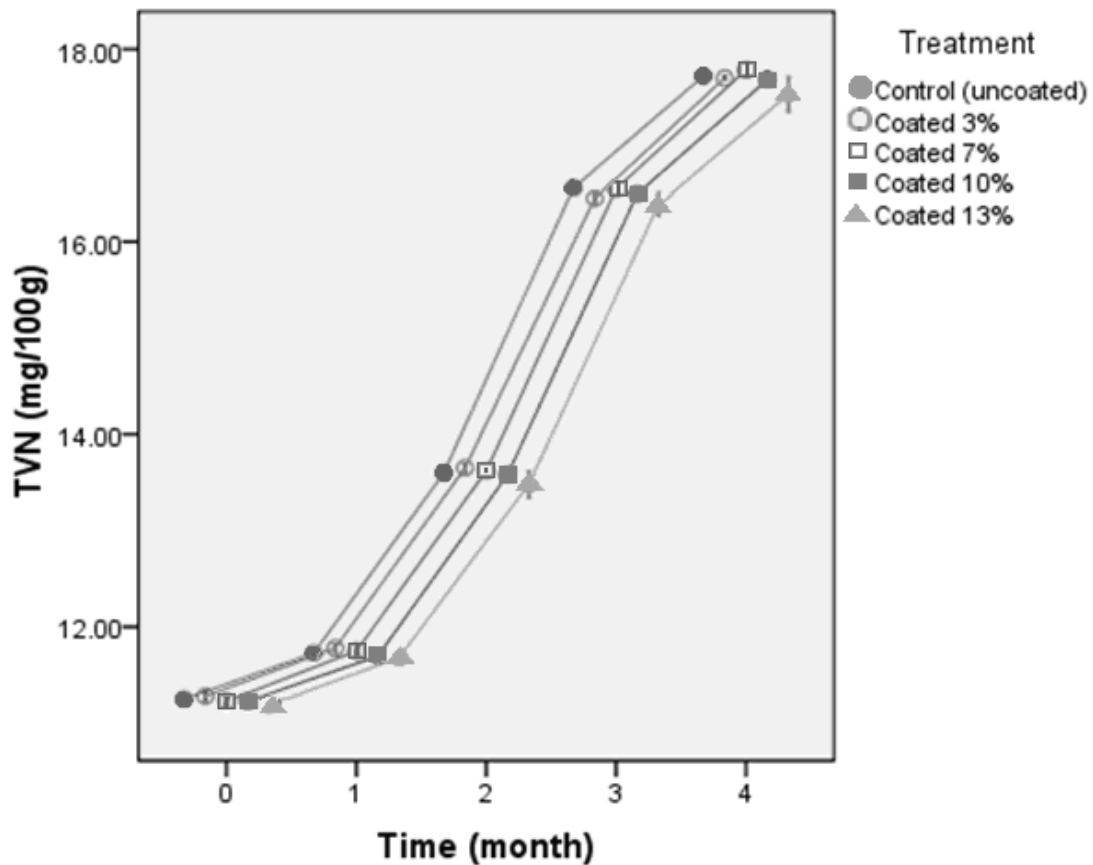
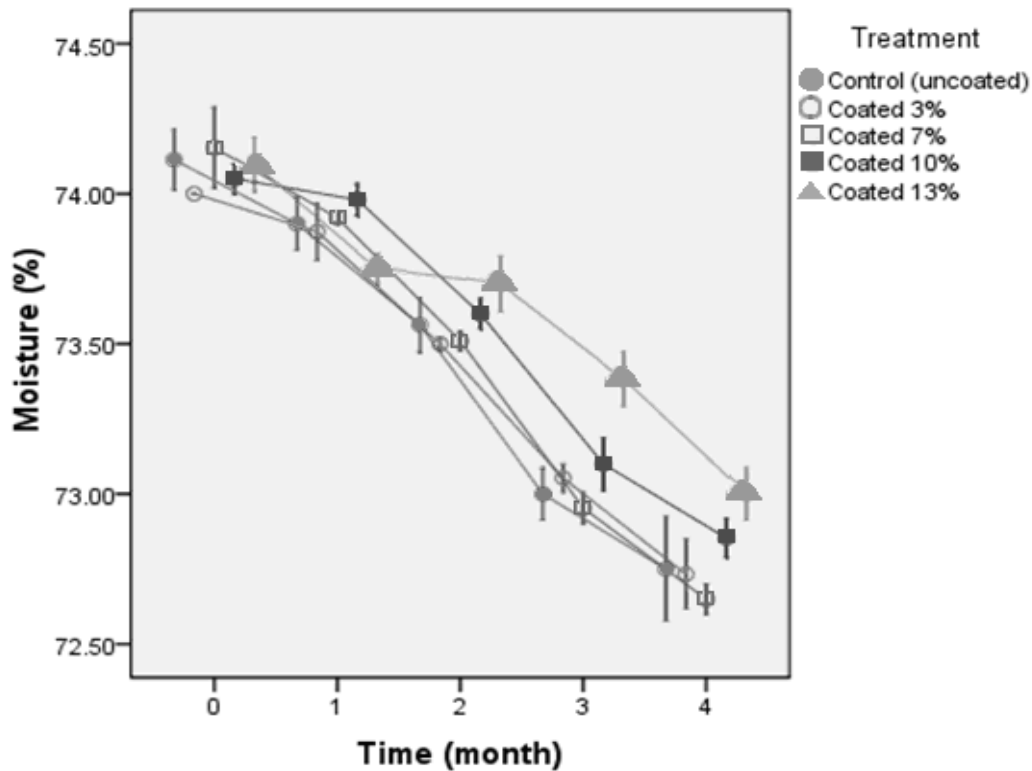


Figure 2: TVN of coated and uncoated samples with whey protein during a 4-month frozen storage

Moisture content in all samples is shown in Fig. 3. Amount of moisture expressible decreased from 74% to 72.75, 72.73, 72.65, 72.85 and 73%, respectively in different treatments (control, coated %3, %7, %10 & %13) at

the storage period ( $p < 0.05$ ). The whey protein-coated samples by 13% concentration showed lower moisture values compared with other samples at months 1, 2, 3 and 4 ( $p < 0.05$ ).



**Figure 3: Moisture of coated and uncoated samples with whey protein during a 4-month frozen storage**

### Discussion

Total microbial count showed increase during 4 months storage suggested that kilka fish still is highly perishable under chilled conditions. The recommended shelf-life of frozen stored fish in the present study was 4 months, if the acceptability limit of 107 CFU/g is applied (Duan *et al.*, 2010). In this study, no significant difference was observed in total microbial count of coated samples Kilka fish which could be due to high value of moisture in product that reduces antioxidant properties of coating (Nortje

*et al.*, 2006). Lack of any significant difference on total microbial count has been previously reported by other authors (e.g., Belgheisi *et al.*, 2008 on fresh mutton). In spite of the fact that edible coatings based on protein have a good gas barrier, however due to polar nature of polypeptidic lattice, it seems that edible coating does not have significant antioxidant properties (in higher relative humidity), so it does not have barrier properties to aerobic organisms (Gennadios, 2002). TVN did not exceed allowable limit in Kilka fish

samples after 4 months, according to Parvaneh, (1998), suggesting that, they are suitable for human consumption. Therefore, it seems that whey protein edible coating did not have significant impact on Kilka fish. The TVN was related to protein breakdown and the observed increases may be attributed to the formation of ammonia or other basic compounds due to microbial activity (Badr, 2004). Therefore, according to the results of the microbial tests, it was expected that TVN does not have, significant difference in samples viewpoint coating. Stuchell *et al.* (1995) described that acetylated monoglyceride edible coating, alone or with isolated whey protein solution was effective in preventing of reduction moisture in salmon fish, up to 42-65%. Sathivel (2005) reported that chitosan edible coating with 1 and 2% concentration was effective in reducing about 50% relative humidity loss compared with the control uncoated fillets of Pink Salmon. Jeon *et al.* (2002) demonstrated that chitosan-coated Atlantic cod and herring had reduced moisture loss. Results of the present study showed that moisture of coated samples with 13% concentration whey protein, had significant differences with other treatments ( $p < 0.05$ ). although moisture content of coated samples with 13% concentration was always less, but as it was expected due to hydrophilic property of whey protein edible coatings. These coatings did not have sufficient barrier properties among moisture.

Therefore, it can be concluded that coated samples with 13% concentration whey protein provided humid surfaces for necessary water activation to microbial growing and between the coated and uncoated samples didn't have any differences. The Control of surface moisture content could significantly reduce the growth of microorganisms and the rate of deteriorative reactions, thereby increasing the storage stability of foods. Edible films and coatings with good water and/or oxygen barrier properties are usually not adequate by themselves to retard microbial growth. Therefore, the incorporation of antimicrobial agents into edible coating formulations is needed to obtain stronger inhibitory effect against microbial growth (Ozdemir & Floros, 2008). As conclusion; it seems that use of whey protein solution with 13% concentration as coating can be effective in reducing moisture loss in coated Kilka fish samples up to 4 month storage.

## Acknowledgment

Special gratitude to the personnel and researchers of the National Fish processing research Center.

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